THE EXTRACTION OF POLYRIBOSOMES FROM LYOPHILIZED CELLS OF *SACCHAROMYCES CEREVISIAE*

by

Robert Ernest Kelley

A Thesis Submitted to the Faculty of the

COMMITTEE ON AGRICULTURAL BIOCHEMISTRY AND NUTRITION

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

1 9 6 8

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Request for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Robert E. Kelley

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Konrad Keck

Konrad Keck
Associate Professor of
Biological Sciences

May 17, 1968

Date

ACKNOWLEDGMENTS

The author expresses sincere appreciation to Dr. Konrad Keck for suggesting and guiding the work represented in this thesis and for his encouragement during the course of research.

Helpful suggestions about the preparation of this thesis were made by Dr. M. Zaitlin and Dr. A. Kemmerer and were gratefully received.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

ABSTRACT

A method is described which permits efficient and reproducible isolation of polyribosomes from lyophilized yeast cells. Rapidly labelled cyctoplasmic messenger RNA has been separated from the rapidly labelled ribosomal precursor RNA. A preliminary study was performed to study the effect of uracil starvation on the polyribosome levels in a uracilless, histidineless yeast mutant.

INTRODUCTION

The cell wall of yeasts is a formidable barrier which may only be disrupted by harsh methods. Most of these methods, such as grinding aqueous suspensions with quartz sand (Chao and Schachman, 1956), or glass beads (Shortman and Fukuhara, 1963), apparently lead to degradation of subcellular components as well as macromolecules, since no high molecular weight rapidly labelled ribonucleic acid (RNA) was isolated. Other techniques involve vigorous shaking of cell suspensions with glass beads (Weil, Bakes, Befort, and Ebel, 1964) or cell disruption by decompression in the French pressure cell (Kihara, Hu, and Halvorson, 1961). Again, these procedures resulted in the failure to isolate high molecular weight RNA fractions or polyribosomes.

Attempts to isolate polyribosomes from cells by grinding with glass beads were partially successful (Marcus, Ris, Halvorson, Brett-hauer, and Bock, 1967). However, the technique resulted in low yields which in addition were strongly dependent upon the length of grinding. deKloet (1966) and Hutchinson and Hartwell (1967) were successful in isolating polyribosomes from lysed spheroplasts of yeast. Unfortunately, the method of spheroplast formation is not applicable to experiments which require cells from the logarithmic growth phase.

A lyophilization technique for the preparation of high molecular weight RNA from intact yeast cells had already been developed (Moore, Schlapfer, Yall, and Keck). Orientation experiments with

lyophilized cell preparations indicated that this technique was also suitable for the isolation of monomers, but polyribosomes were not found. In this study, a lyophilization technique for the preparation of polyribosomes from yeast has been developed.

The double auxotrophic yeast strain (uracilless and histidineless) used in this study is insensitive to Actinomycin D; in the absence of uracil, RNA synthesis is decreased to 3% of the normal rate after 90 minutes. Nevertheless, the rate of protein synthesis continues at only slightly reduced rates for 10 hours (Bullaro). Since the existence of a long-lived messenger in this system was suspected, an attempt was made to study the effect of uracil starvation on the polyribosome levels in intact cells using the lyophilization technique.

MATERIALS AND METHODS

I. Yeast Strain

Saccharomyces cervisiae, strain S2112D ($a\text{-}ur_4$), a uracil and histidine requiring mutant, was provided by Dr. R. K. Mortimer, University of California, Berkeley, California. This strain was used in all the experiments described in this manuscript.

II. Media

All cultures of yeast were grown in media designated C.R.M., complete Roman media (Roman, 1956). In labelling experiments, C.R.M. minus adenine, uracil, and histidine was employed and termed "D" medium. When the labelled material was adenine, 10 mls. of a stock solution of 1 mg/ml uracil, and 10 mls. of a stock solution of the same concentration of histidine were added to 1 liter of "D" medium. In spheroplast experiments, the media used for resuspension of the spheroplasts was designated YM-5 (Hartwell, 1967).

III. Chemicals

Sodium dodecyl sulfate (SDS) and sodium deoxycholate (DOC) were obtained from Matheson Coleman and Bell (Norwood, Ohio); Atlas Brij 58 was a gift of Dr. M. Zaitlin. All radioactive materials were the products of New England Nuclear (Boston, Mass.). Cycloheximide (Actidione) and bovine pancreatic ribonuclease (RNase), 5X crystalized, A grade, were purchased from CalBiochem (Los Angeles, Calif.). Glusulase, a commercial preparation of snail digestive juice, was purchased

from Endo Laboratories (Garden City, N. J.) and contained 170 mg of protein per ml. 2,5-bis(2-(5-tert-Butyl)(-Benzoazolyl))-Thiophene (BBOT) is a product of the Packard Instrument Co. (Downers Grove, Ill.), and Ethylene glycol monoethyl ether (cellosolve) was obtained from Eastman Kodak Co. (Rochester, N. Y.).

IV. Radioactive Labelling

Unless otherwise indicated, all labelling was performed in "D" + uracil + histidine to which adenine-C^{14} or adenine-H^3 was added. Labelling was carried out during a resuspension phase to be described later. Labelled samples were read in a Beckman LS II Liquid Scintillation Spectrometer. A scintillation fluid consisting of 4g BBOT, 80g Naphthalene, 400 mls Methylcellosolve, and 600 mls of Toluene was used (BBOT - cocktail). Aqueous samples of 0.5 ml were added to 12 mls of the BBOT - cocktail for counting.

V. Sucrose Gradient Centrifugation

RNA sedimentation analyses were performed using a linear sucrose gradient of 25 - 5%. Gradients were prepared in a 0.1M EDTA, 0.1M KAc buffer of pH 5.9. Nine 0.5 ml aliquots were layered by pipette into 5 ml centrifuge tubes. Samples of 0.2 ml were layered over the gradients and centrifuged in the SW - 39 Spinco rotor for 5 hours at 39,000 rpm at $5^{o}C$. Ribosome preparations were extracted with a 0.01M Tris-HCL, 0.01M $MgSO_4$, 0.1M KCl buffer of pH 7.6 containing 0.5% w/v DOC (TMKD buffer). Linear sucrose gradients of 30 - 10% (4.5 mls), were prepared in the same buffer minus DOC; 0.2 ml samples were layered over each

gradient and centrifuged at 39,000 rpm in the Spinco SW - 39 rotor for 1-1/2 hours at 5°C. Polyribosome isolation experiments employed a buffer consisting of 0.01M Tris-HCl, 0.0015M $MgSO_4$, and 0.1M NaCl at a pH of 7.4, which contained 50 ug/ml bentonite (TMNB). TMNB lysing and extracting buffer contained 0.5% w/v Brij and 0.5% w/v DOC. Linear sucrose gradients of 30 - 10% were prepared in TMNB minus the detergents. Samples of 0.2 - 0.3 ml were layered over the gradients and centrifuged at 33,000 rpm in the Spinco SW - 39 rotor at 5°C for 1 hour.

VI. Yeast Culture Preparations

Yeast cultures were grown sterily in cylinders containing 1 liter of C.R.M. with forced aeration (30 ml/min.) at 30°C in a constant temperature room. The cultures were harvested by Millipore filtration (pore size = 0.64 u) during the log phase of growth and at concentrations ranging from 6×10^6 cells/ml to 20×10^6 cells/ml. All cultures were inspected by phase microscopy for bacterial contamination before use. The collected cells were resuspended in 50 mls of C.R.M. When an RNA label was employed, 50 mls of "D" + uracil + histidine were used. Aeration was allowed to proceed for 30 - 60 minutes in a 30°C water bath. If the culture was to be used for isolation of polyribosomes from lyophilized cells, cycloheximide, an inhibitor of protein synthesis, was added to the suspension to a final concentration of 100 ug/ml; aeration was continued for another 10 minutes. The suspension was then poured into 40 ml conical centrifuge tubes containing 1/2 volume of crushed ice. The cells were pelleted by centrifugation at 3200 rpm for 10 minutes at 3°C in a PR-2 International refrigerated centrifuge.

VII. Spheroplast Preparations

The collected cells were washed by resuspension in several volumes of ice-cold distilled water and recentrifuged. Spheroplast formation and polyribosome isolation were then carried out according to the method of Hutchinson and Hartwell (1967). In principle, the washed cells were resuspended in 1M sorbitol, making a final concentration of 2×10^8 cells/ml. Enough glusulase was added to the cell suspension to give a final concentration of 1% v/v and the mixture was incubated in a 30°C water bath for 90 minutes. The spheroplasts were collected by centrifugation at 1500 rpm for 10 minutes and resuspended in equal amounts of YM-5. Incubation was continued in the 30°C water bath for 2 hours. Cycloheximide was added to the suspension to a final concentration of 100 ug/ml and after 10 minutes, the spheroplasts were rapidly cooled in an ice-water bath. The spheroplasts were pelleted and the supernatant decanted. A minimal amount of TMNB lysing buffer was added to the pellet and extraction was carried out in an ice bath for 5 minutes. The extraction mixture was then given a 15,000 rpm spin for 10 minutes in the Spinco L 40 rotor. The supernatant was collected and 0.2 - 0.3 ml samples were layered over the 30 - 10% sucrose gradient. After sedimentation, 2 drop fractions were collected from the bottom of the centrifuge tubes and diluted with 2.5 mls of distilled water for optical density measurements.

VIII. Ground Cell Preparations

All steps described below were carried out in a 5°C cold room. The pellets were collected after draining and a few drops of TMNB

extracting buffer were added to each tube. A paste was prepared by adding either 240 mesh silica powder or 150 mesh carborundum powder to the pellet. A small amount of the paste was placed between two heavy glass plates and ground for 1 minute under approximately 20 lbs/in^2 pressure. After grinding, the material was collected on the edge of a razor blade and washed into 1.5 mls of ice-cold TMNB extracting buffer contained in a watch glass. This process was repeated until all the paste had been treated. The extract was collected by Pasteur pipette and transfered to 12 ml centrifuge tubes. Cell debris was removed by centrifugation at 15,000 rpm for 10 minutes. After sucrose gradient centrifugation, 2 drop fractions were collected from the bottom of the tube and diluted with 2.5 mls of distilled water. Optical density readings were performed by using a sequential sampling device. If a label had been used, 0.5 ml aliquots were removed for scintillation counting as described.

IX. Lyophilized Cell Preparations

After pelleting, the cells were collected and placed directly into thick walled glass vials. The cells were frozen onto the walls of the vials by rolling on dry ice. When freezing was complete, the vials were connected to the lyophilizer and the cells lyophilized do dryness. Two grams of glass beads (3mm diameter) were added to each vial and the vials were placed in a -20°C freezer for 1.5 hours. The vials were then placed in a Mickle Tissue Disintegrator (Brinkman Instruments, Westbury, N.Y.) and shaken for 45 seconds at maximum amplitude. In ribosome and polyribosome isolations, 1.5 mls of ice-cold TMKD or TMNB

extracting buffer was added to the broken cell preparation and shaken vigorously. Extraction was allowed to continue for 10 minutes in an ice bath; the homogenate was then collected and treated in the same manner as described for ground preparations.

Broken cell preparations to be used for total RNA extraction experiments were treated in the following manner: 15 mls of an 85% phenol solution (12.75 mls of redistilled phenol + 2.25 mls of water) and 15 mls of a bentonite-SDS slurry (80 mls of neutral 0.1M EDTA + washed bentonite + 5% w/v SDS) were mixed and 1.5 mls of this mixture added to the vial. The vial was shaken and the contents transfered to the remaining phenol-SDS-bentonite slurry and stirred overnight on a magnetic mixer in the cold (5°C). The mixture was removed and centrifuged, and the aqueous layer transferred to visking tubing. Dialysis was carried out for 8 hours against 0.1M KAc solution, pH 6.8. The potassium dodecyl-sulfate precipitate formed during dialysis was removed by centrifugation at 3200 rpm for 10 minutes. The supernatent was removed and 3 volumes of ice-cold ethanol (95%) added. Precipitation was complete after 10 hours at -20°C. The RNA precipitate was pelleted, resuspended in one ml of water, and sucrose gradient sedimentation performed as described above.

X. Uracil Starvation Experiments

Cells from 500 mls of a liter culture were collected and transferred sterily to one liter of "D" + adenine + histidine. Aeration was continued for 5 hours; then, the cells were harvested on a millipore filter and resuspended in 50 mls "D" + adenine + histidine. Cycloheximide was added during the last 10 minutes of the resuspension period to

a final concentration of 100 ug/ml. The cells were treated in the same manner described above for the isolation of polyribosomes from lyophilized cells. The remaining 500 mls of the original liter culture were prepared immediately for polyribosome isolation from lyophilized cells.

RESULTS

I. Preparation of the Polyribosomes from Spheroplasts

To establish the proportion of polyribosomes to monomers in an undegraded preparation, i.e., to obtain a reference level for the comparison of polyribosome preparations isolated by the various methods investigated, polyribosomes were isolated from spheroplasts as described by Hutchinson and Hartwell(1967). The results of a typical sedimentation profile of polyribosomes isolated from spheroplasts, and the effect of 5 ug/ml RNase(5°C for 10 minutes) on the polyribosome-monomer distribution is shown in Figure 1. The untreated preparation exhibits a U.V. profile similar to those reported in the literature. As expected, RNase treatment of the spheroplast extract results in a drastic shift in the polyribosome-monomer distribution toward the monomers.

II. Preparation of Polyribosomes from Ground Cells

Since it was reported that undegraded polyribosomes had been prepared by grinding cell suspensions with glass beads with mortar and pestle (Marcus, Ris, Halvorson, Bretthauer, and Bock, 1967), a similar experiment was performed using the a-ur_4 yeast mutant. It seems that the cell wall of a-ur_4 is resistent even to grinding with glass beads, since less than 3% of the cells were broken. When the cells were ground with silica powder or carborundum powder between heavy glass plates, much greater cell rupture was obtained. In general, there

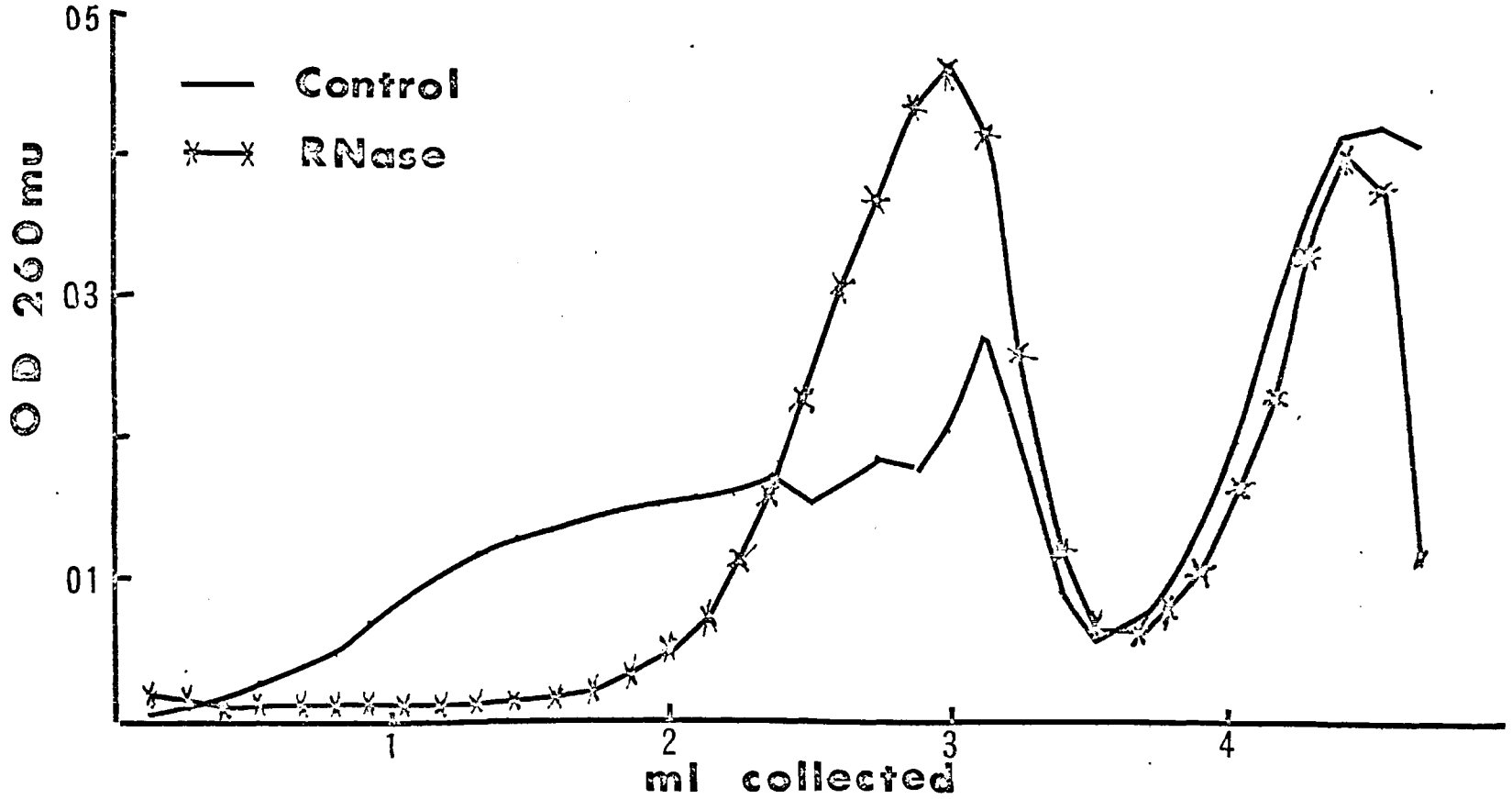

FIG. 1. Polyribosome Isolation from Spheroplasts and the Effect of RNase

were wide fluctuations in the yields and the degree of degradation of polyribosomes isolated in these experiments (Figure 2), and the techniques were not pursued further.

III. Isolation of 80S Ribosomes from Lyophilized Cells

First attempts to isolate polyribosomes from lyophilized cells resulted in a consistent recovery of 50 - 70% of the 80S ribosomes present in the intact cell (as determined from hot-TCA extractable RNA, determined by the orcinol method; Figure 3). The failure to isolate polyribosomes was initially suspected to be due to nuclease activity which would not be encountered during the phenol-extraction of total RNA. This possibility seems to be unlikely since it was found that the addition of RNase inhibitors such as polyvinylsulfate (Fellig and Wiley, 1959) or bentonite (Brownhill, Jones, and Stacey, 1959) to the extracting buffers and to the sucrose gradients failed to yield polyribosomes.

IV. Isolation of Polyribosomes from Lyophilized Cells

In the method described for the isolation of polyribosomes from spheroplasts, cycloheximide is added to the incubation mixture a few minutes before the spheroplasts are cooled and lysed. This drug was found to inhibit protein synthesis (Siegel and Sisler, 1964a, b, 1965; Bennett, Ward, and Brockman, 1965; Lin, Mosteller, and Hardesty, 1966; Morris, 1966) and to cause the stabilization of polyribosomes (Wettstein, Noll, and Penman, 1964; Stanner, 1966; deKloet, 1966) in yeast and other systems. It was noted that when the drug was accidently omitted, greatly reduced polyribosome yields were obtained. With this

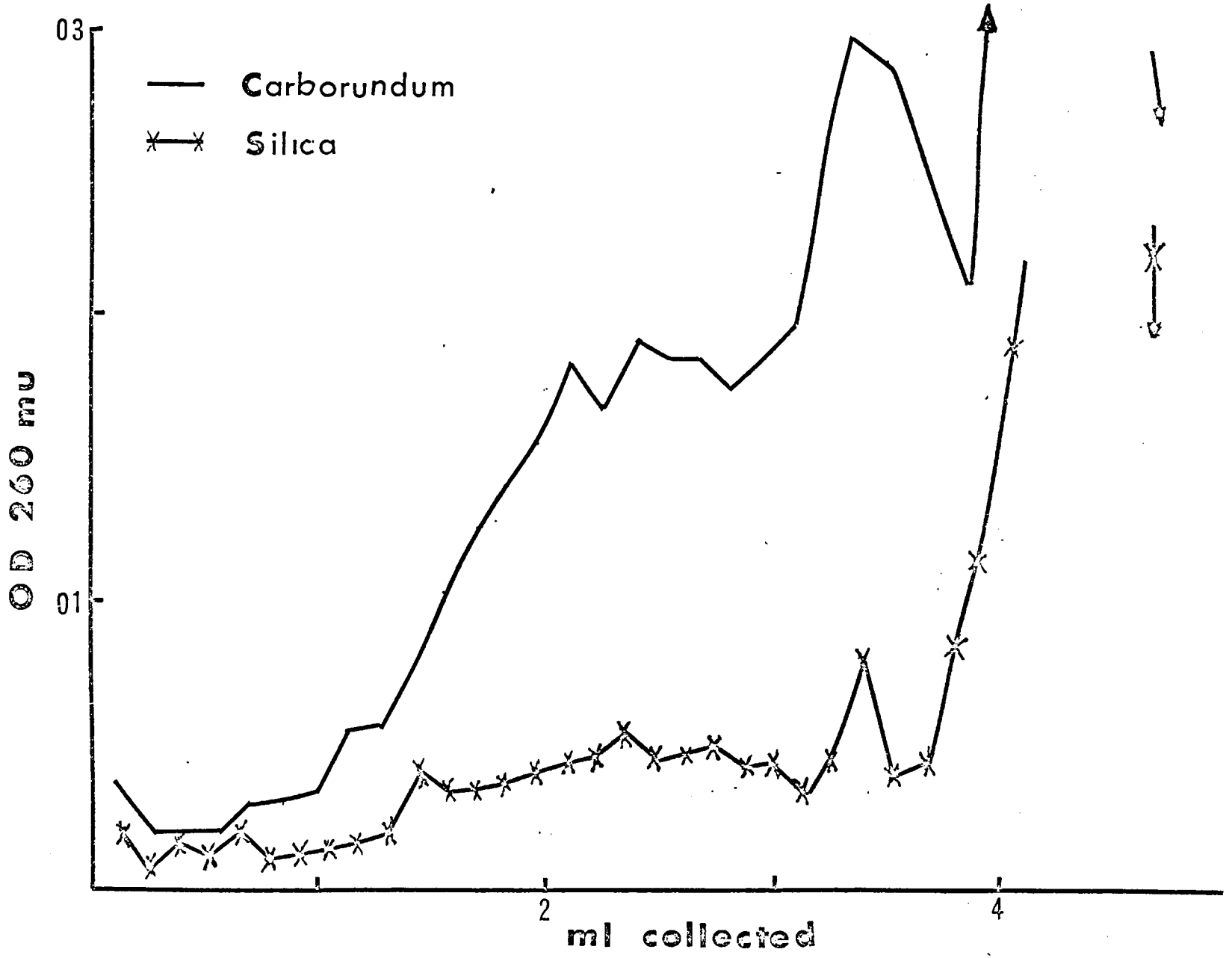

FIG. 2. Polyribosomes from Ground Cells

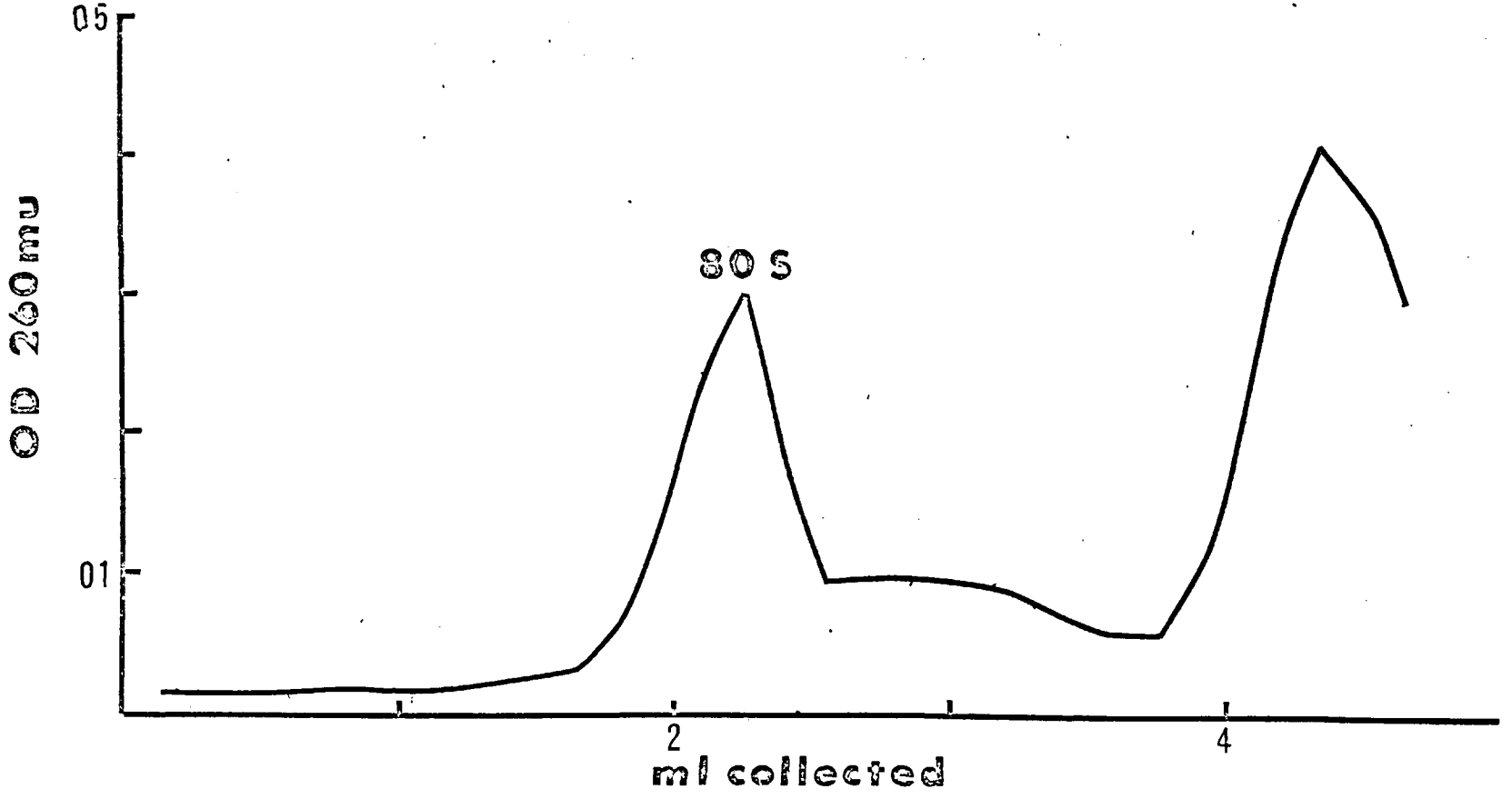

FIG. 3. Ribosome Isolation from Lyophilized Cells

in mind, the effect of cycloheximide on the preparation of polyribosomes from lyophilized cells was investigated. It was found that polyribosomes could be isolated from cells that were exposed to cycloheximide prior to lyophilization. The effect of 5 ug/ml RNase (5°C for 10 minutes) was similar to that observed in spheroplast preparations (Figure 4). Furthermore RNA extracted from pooled polyribosome fractions labelled in vivo for 1 hour with uridine-H^3, was found to contain not only labelled 28S and 18S ribosomal RNA, but also a 15S RNA fraction with a high specific activity (cpm/O.D.) as shown in Figure 5. This 15S RNA component is presumably messenger RNA (m-RNA); the sedimentation value of 15S agrees favorably with values obtained by other investigators in similar experiments (Wilson and Hoagland, 1967; Moore and Keck).

V. The Effect of Uracil Starvation on Polyribosome Levels in a-ur_4 Cells

The technique of polyribosome isolation from lyophilized cells was applied in a preliminary study of the effect of uracil starvation on polyribosome levels in a-ur_4 cells. In the absence of uracil, a-ur_4 cells show only 3% of the normal rate of RNA synthesis as determined by adenine-C^{14} uptake. As shown in Figure 6, reduced polyribosome levels were found in cells starved for uracil, while normal polyribosome levels were present in the non-starved control cells. The results suggest that in the absence of m-RNA synthesis, caused by uracil starvation, polyribosomes disappear due to decay of the m-RNA which existed prior to uracil starvation.

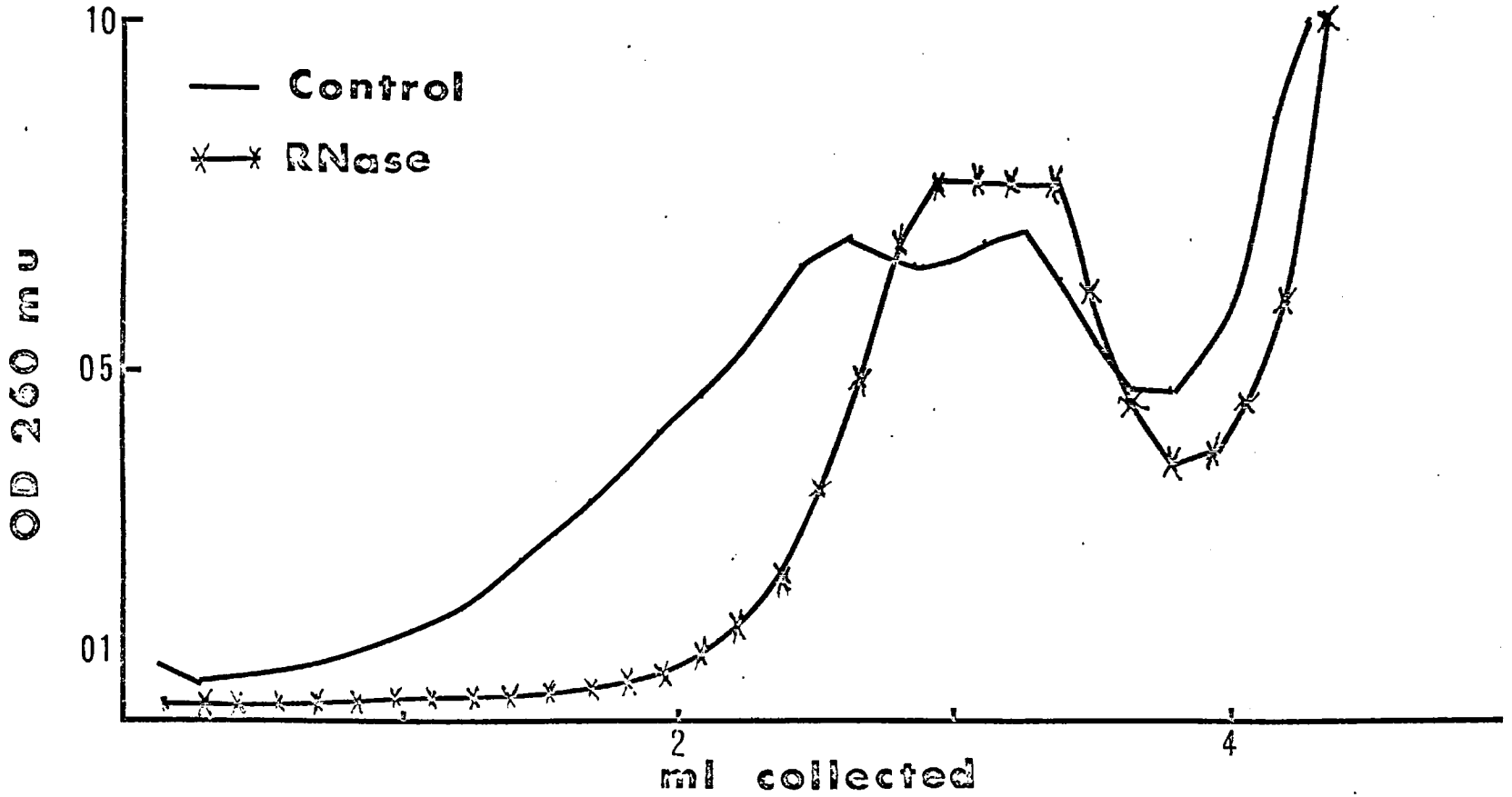

FIG. 4. Polyribosomes from Lyophilized Cells and the Effect of RNase

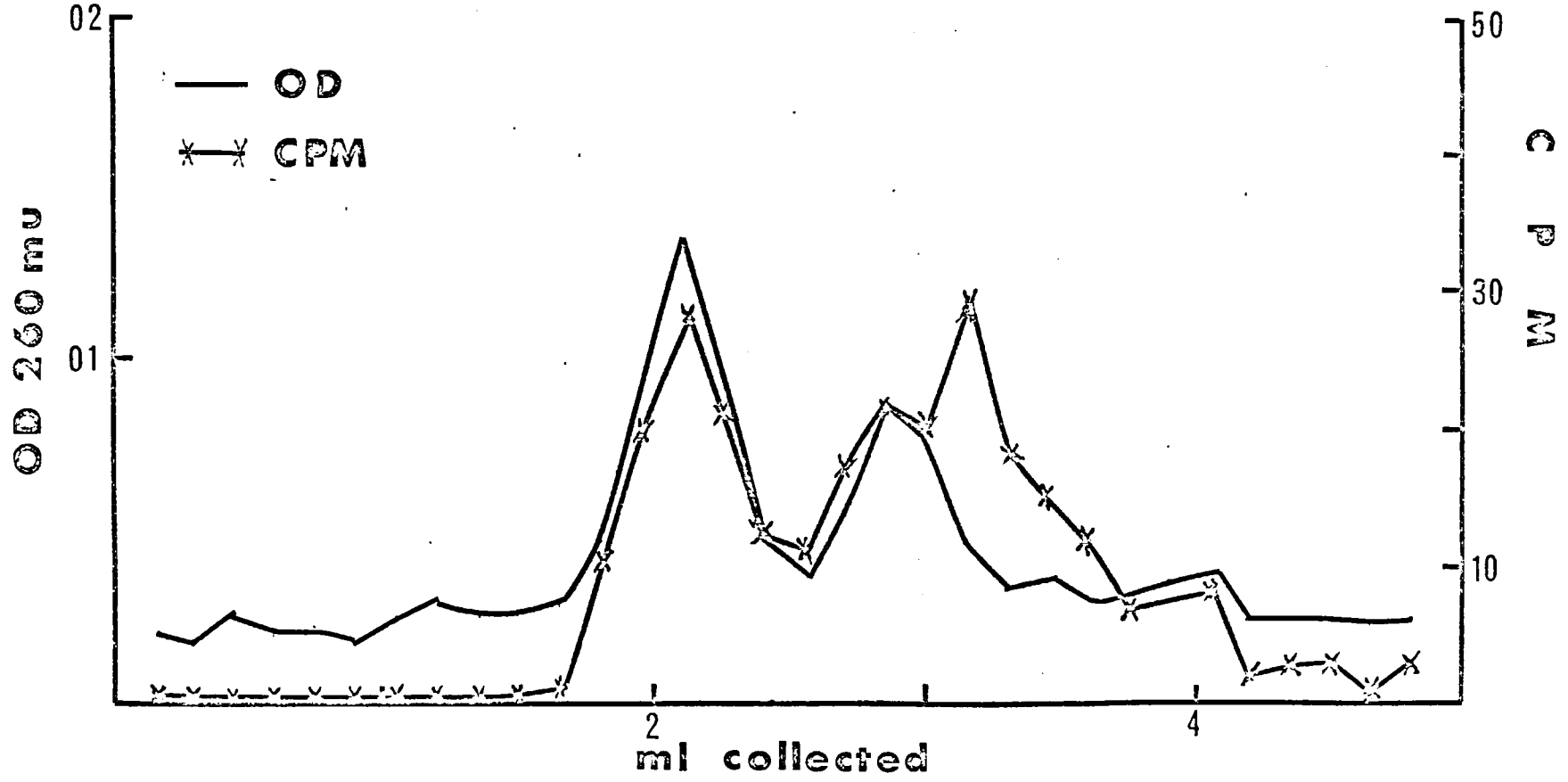

FIG. 5. RNA from Pooled Polyribosome Fractions from Lyophilized Cells

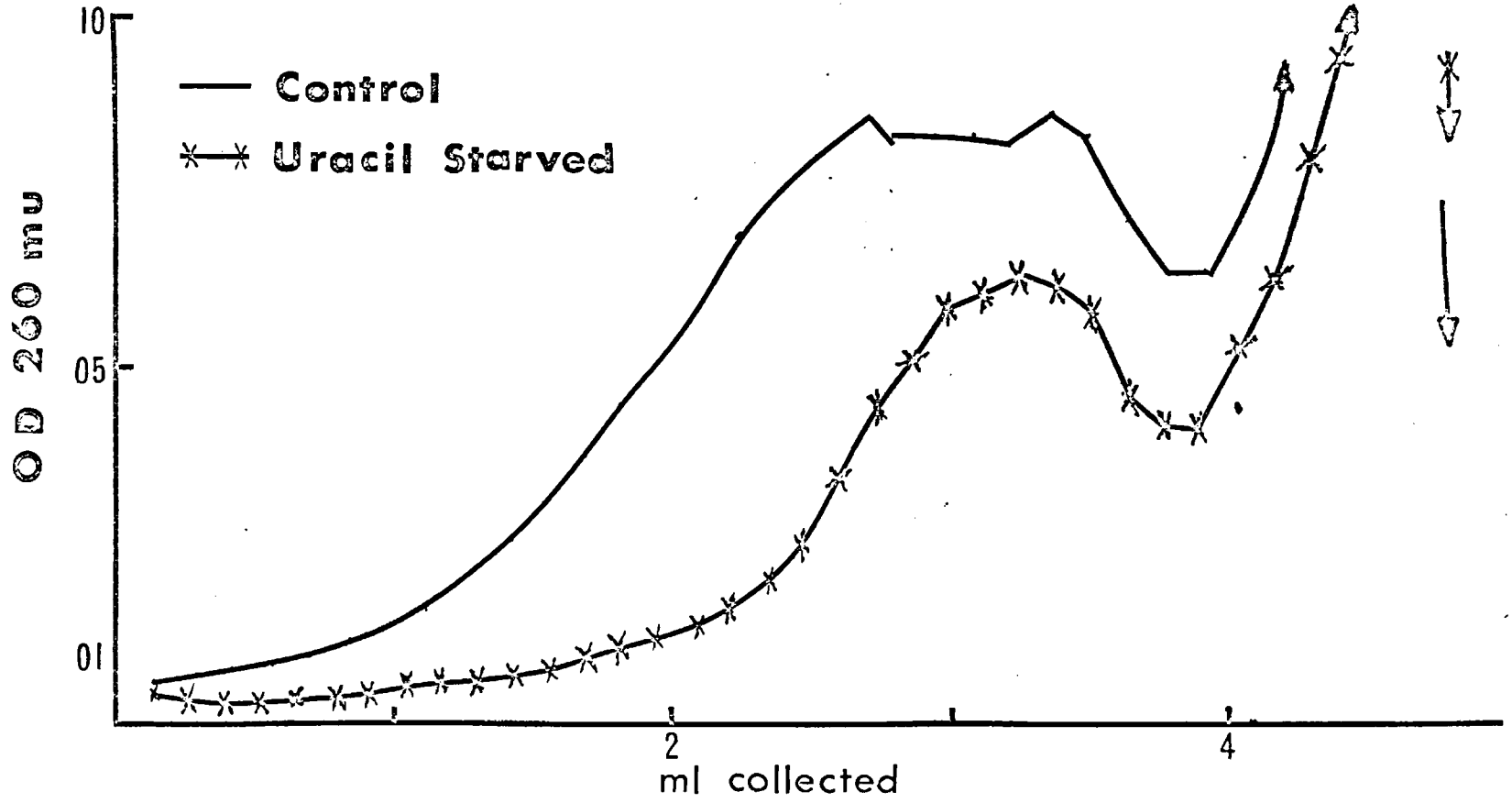

FIG. 6. Effect of Uracil Starvation on a-ur$_4$ Cells' Polyribosome Levels

VI. The isolation of Total RNA from Lyophilized Cells and the Failure to Separate m-RNA from Ribosomal Precursor RNA

The lyophilization technique was originally developed for the preparation of total RNA extracts from intact cells (Moore, Schlapfer, Yall, and Keck). When lyophilized cells are extracted with a phenol-SDS-bentonite suspension, undegraded rapidly labelled high molecular weight RNA is extracted (Figure 7). In a three minute pulse experiment with adenine-C^{14}, the specific activities (cpm/O.D.) of the 28S and 18S ribosomal RNA components are low, and aside from the low molecular weight t-RNA, high specific activities were found in a heterogenous fraction which sediments with 2 peaks at approximately 30 and 45S. This rapidly labelled, high molecular weight RNA was tentatively identified as ribosomal precursor RNA.

VII. Attempt to Separate the Rapidly Labelled Ribosomal Precursor RNA and Rapidly Labelled m-RNA

A comparison of the results obtained with phenol-extracted total RNA preparations and with RNA extracted from pooled polyribosomal material, shows that the labelled 15S fraction is not present in the phenol-extracted RNA. It seemed possible that the 15S fraction extracted from polyribosome preparations was partially degraded high molecular weight m-RNA molecule which in total RNA extractions sediments together with the 30 - 45S ribosomal precursor RNA.

An attempt was made to separate the ribosomal precursor RNA from the high molecular weight m-RNA. Since ribosomal precursor RNA was reported to have a significant amount of secondary structure, it

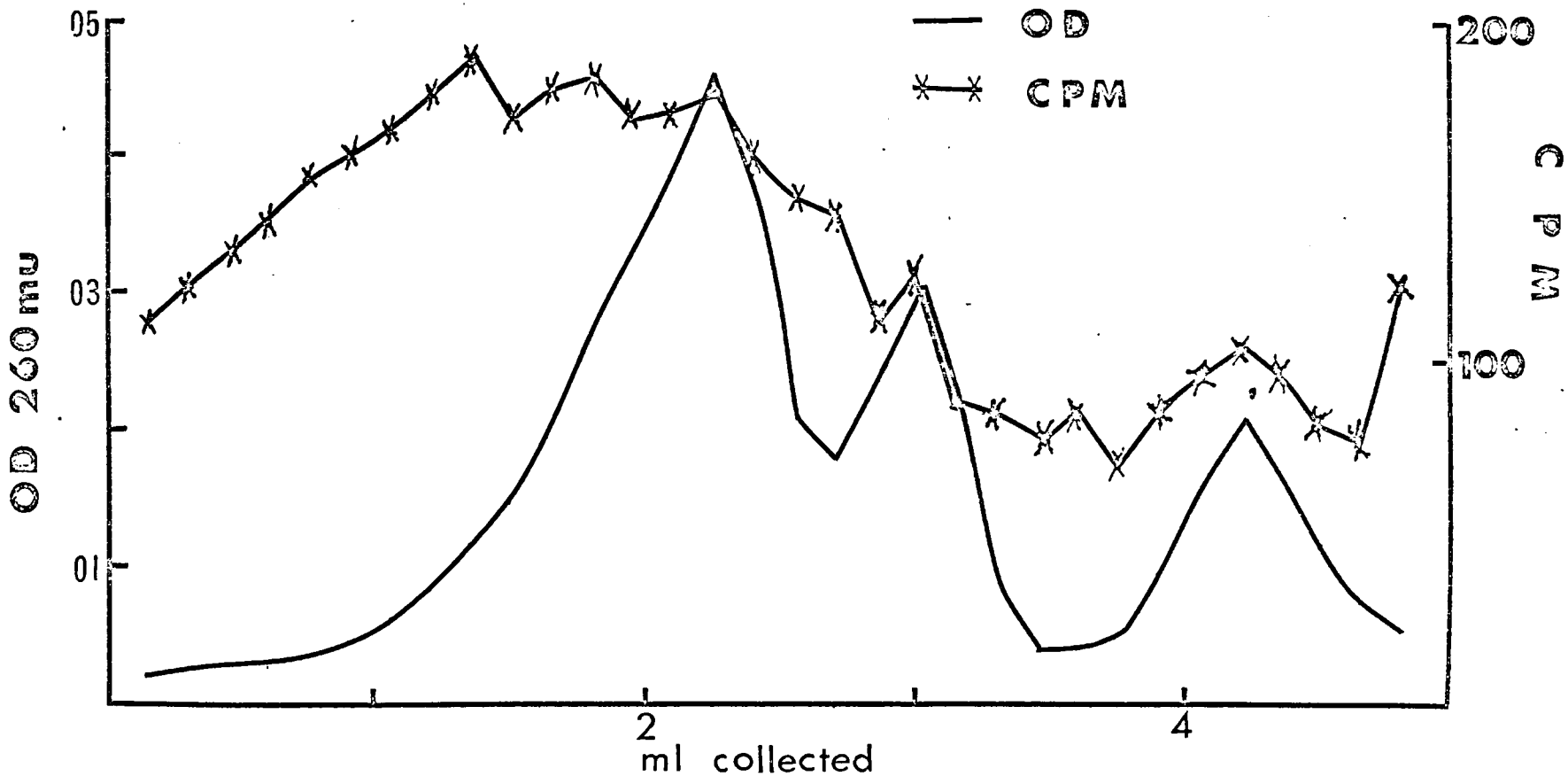

FIG. 7. Total Phenol-Extracted RNA from Lyophilized Cells

was thought that heating this RNA fraction in the presence of HCHO would result in the stabilization of the molecule in the unfolded configuration. m-RNA on the other hand is presumed to be a random coil and treatment with heat and HCHO should have no effect on the structure of the molecules. Sedimentation analysis might then show that the unfolded ribosomal precursor RNA now sediments with a reduced sedimentation coefficient, while the m-RNA molecules retain the 30 - 40S value.

The results of such an experiment are shown in Figure 8. As predicted, the ribosomal precursor RNA sediments with a lower sedimentation coefficient. Significant levels of label are no longer found in the 30 - 45S region suggesting that no significant amounts of m-RNA are contained in the 30 - 45S region, and that a separation of m-RNA from the rapidly labelled ribosomal precursor RNA in total RNA extracts is not feasible. Therefore, only cytoplasmic m-RNA contained in polyribosomes can be recognized in yeast at the present time.

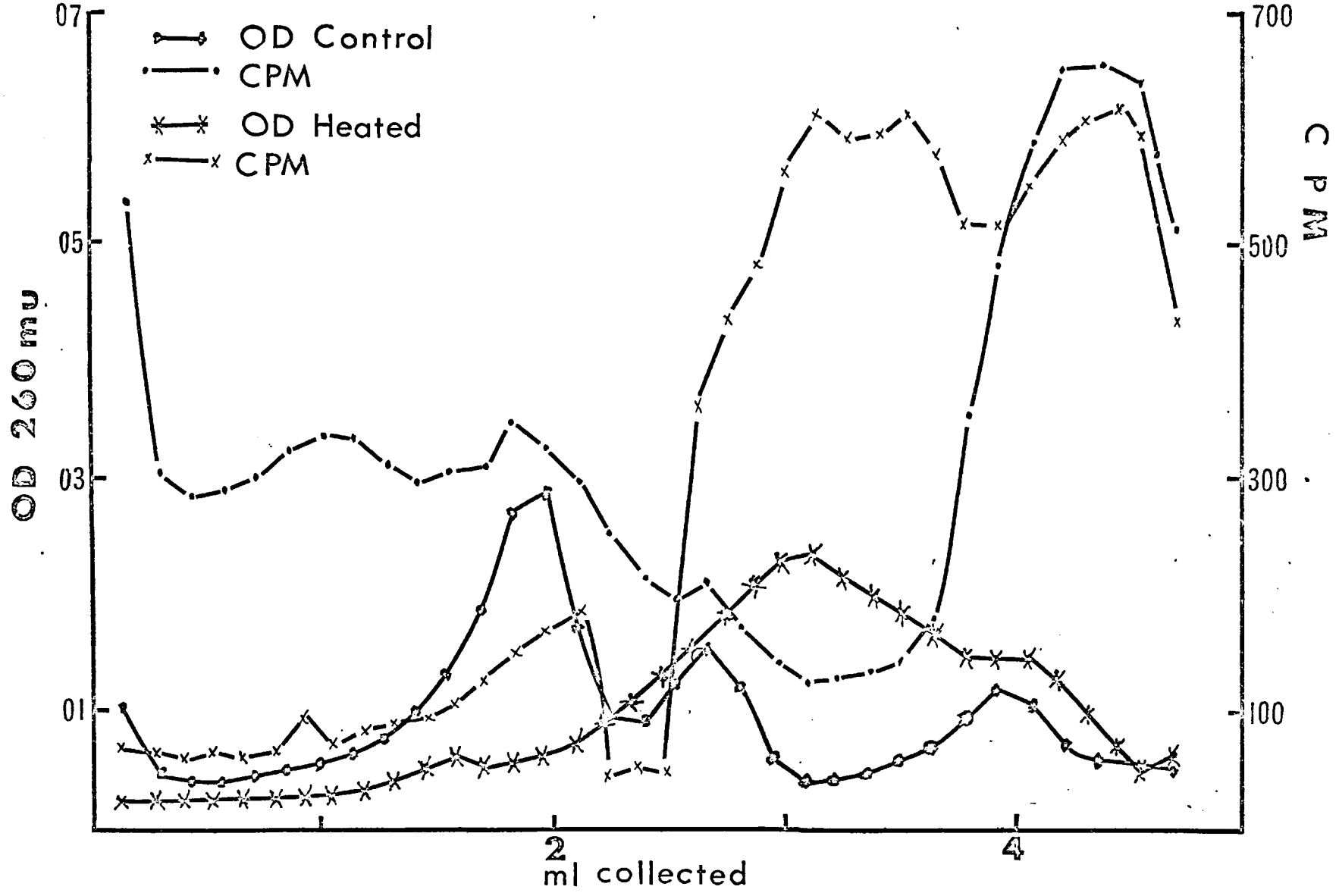

FIG. 8. RNA Heating Experiment

DISCUSSION

I. Polyribosome Isolation from Lyophilized Cells

A possible explanation for the distortion observed in the polyribosome profiles of lyophilized cells is that during lyophilization, a large amount of non-specific binding of proteins and other material to the ribosomes and polyribosomes occurs. Preliminary results with buffers of high ionic strength indicate that the dissociation of adsorbed material can not be achieved with 0.5M NaCl. An alternative explanation is that in defined minimal media, such as C.R.M., the normal distribution of polyribosome size is smaller than that found when cells are grown in an enriched media such as YM-5. The effects of media on polyribosome size may be related to the initial frequency of binding of monomers to the m-RNA molecule, the rate of initiation of polypeptide formation and the translation of the m-RNA molecule (Trakatellis, Montjar, and Axelrod, 1965; Conconi, Banks, and Marks, 1966).

II. Effect of Uracil Starvation on a-ur_4 Cells

An attempt was made to obtain evidence of a long-lived messenger RNA molecule in a-ur_4 yeast cells. The results of a uracil starvation experiment indicate significant loss of polyribosomal material in uracil starved cells, probably due to the decay of m-RNA in the absence of *de novo* RNA synthesis. The results can not rule out the existence of a long-lived m-RNA species in a-ur_4 cells, but do suggest that under the conditions tested, the majority of m-RNA is degraded and no longer

present after six hours. An alternative suggestion is that the m-RNA molecule is not degraded and that the lack of polyribosomes is due to an inhibition of monomer binding. Such a reversible dissociation has been reported for a variety of systems which have been treated with agents known to interfere with the energy metabolism of the cell, such as ethionine, DNP, NaF, azide, etc. (Baglio and Farber, 1965; Conconi, Banks, and Marks, 1966).

III. Lyophilization as a Technique for the Isolation of Various RNA Fractions

From the experiments cited, the technique of lyophilization has been found to be suitable for the analytical separation of labile RNA fractions obtained from lyophilized yeast cells. During cell disruption, only a minimal amount of mechanical or enzymatic degradation of the RNA fractions could have occurred. The advantage of this technique becomes apparent when it is necessary to work with cells grown under physiological conditions not applicable to spheroplast preparations, or when very small samples are processed. One of the future applications of the technique of polyribosome isolation from lyophilized cells is the isolation and characterization of the rapidly labelled m-RNA fraction free from ribosomal precursor RNA fractions.

LIST OF REFERENCES

Baglio, C. M. and E. Farber, "Correspondence between Aggregation Patterns in Rat Liver Homogenates and in Electron Micrographs Following Administration of Ethionine," J Mol. Biol. 12/466 (1965).

Bennett, L. L., V. L. Ward, and R. W. Brockman, "Inhibition of Protein Synthesis in vitro by Cycloheximide and Related Glutarimide Antibiotics," Biochim. Biophys. Acta 103/478 (1965).

Brownhill, T. J., A. S. Jones, and M. Stacey, "The Inactivitation of Ribonucleases During the Isolation of Ribonucleic Acids and Ribonucleoproteins from Yeast," Biochem. J. 73/434 (1959).

Bullaro, J., PhD. dissertation, in preparation.

Chao, F. C. and H. K. Schackman, "The Isolation and Characterization of a Macromolecular Ribonucleoprotein from Yeast," Arch. Biochem. Biophys. 61/220 (1956).

Conconi, F., A. Banks, and P. A. Marks, "Polyribosomes and Control of Protein Synthesis: Effects of Sodium Fluoride and Temperature in Reticulocytes," J. Mol. Biol. 19/525 (1966).

de Kloet, S. R., "RNA Synthesis in Yeast. The Effect of Cycloheximide on the Synthesis of RNA in Saccharomyces carlsbergensis," Biochem. J. 99/566 (1966).

Fellig, J. and C. E. Wiley, "The Inhibition of Pancreatic Ribonuclease by Anionic Polymers," Arch. Biochem. Biophys. 85/313 (1959).

Hartwell, L. H., "Macromolecular Synthesis in Temperature Sensitive Mutants of Yeast," J. Bacteriol. 93/1662 (1967).

Hutchinson, H. T. and L. H. Hartwell, "Macromolecule Synthesis in Yeast Spheroplasts," J. Bacteriol. 94/1697 (1967).

Kihara, H. R., A. S. L. Hu, and H. O. Halverson, "The Identification of a Ribosomal-Bound β-Glucosidase," Proc. Nat'l Acad. Sci., U.S., 47/4891, 1961.

Lin, S. Y., R. D. Mosteller, and J. Hardesty, "Mechanism of NaF and Cycloheximide Inhibition of Hemaglobin Biosynthesis in the Cell-free Reticulocyte System," J. Mol. Biol. 21/51 (1966).

Marcus, L., R. D. Ris, H. O. Halvorson, R. K. Bretthauer, and R. M. Bock, "Occurrence, Isolation, and Characterization of Polyribosomes in Yeast," J. Cell. Biol. 34/505 (1967).

Moore, K. E. and K. Keck, in preparation.

Moore, K. E., T. Schlapfer, I. Yall and K. Keck, unpublished results.

Morris, I., "Inhibition of Protein Synthesis by Cycloheximide in Chlorella," Nature 211/1190 (1966).

Roman, H. L., "A System Selective for Mutations Affecting the Synthesis of Adenine in Yeast," Compt. Rend. Lab. Carlsberg. Ser. Physiol. 26/299 (1956).

Siegel, M. R. and H. D. Sisler, "Site of Action of Cycloheximide in Cells of Saccharomyces pastorianus. I. Effect of the Antibiotic on Cellular Matabolism." Biochim. Biophys. Acta 87/70 (1964).

_____ and _____, "Site of Action of Cycloheximide in Cells of Saccharomyces pastorianus. II. The Nature of Inhibition of Protein Synthesis in a Cell-free System," Biochim. Biophys. Acta 87/83 (1964).

_____ and _____, "Site of Action of Cycloheximide in Cells of Saccharomyces pastorianus. III. Further Studies on the Mechanism of Resistance in the Saccharomyces Species." Biochem. Biophys. Acta 103/558 (1965).

Shortman, K. and H. Fukuhara, "Metabolism of RNA During Respiratory Adaptation of Yeast," Biochim. Biophys. Acta 76/501 (1963).

Stanner, C., "The Effect of Cycloheximide on Polyribosomes from Hamster Cells," Biochem. Biophys. Res. Commun. 24/758 (1966).

Trakatellis, A. C., M. Montjar, and A. E. Axelrod, "Turnover of Messenger RNA and Protein Biosynthesis in Mouse Mammary H2712," Biochim. 4/1678 (1965).

Weil, J. H., J. Bakes, N. Befort, and J. P. Ebel, "Contributur a L'Etude de RNA Messager de la Levure," Bull. Soc. Chim. Biol., 46/1073, 1964.

Wettstein, F., H. Noll, and S. Penman, "Effect of Cycloheximide on Ribosomal Aggregates Engaged in Protein Synthesis in vitro," Biochim. Biophys. Acta 87/525 (1964).

Wilson, S. H. and M. B. Hoagland, "Physiology of Rat-Liver Polyribosomes. I. Stability of Messenger RNA and Ribosomes." Biochem. J. 103/556 (1967).